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By:

Nancy Foster

PATENT

Customer Number 22,852

Attorney Docket No. 4121.0116-01000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Holly H. HOGREFE et al.

Serial No.: 08/957,709

Filed: October 24, 1997

For: POLYMERASE ENHANCING  
FACTOR (PEF) EXTRACTS, PEF  
PROTEIN COMPLEXES, ISOLATED  
PEF PROTEIN, AND METHODS  
FOR PURIFYING AND  
IDENTIFYING THEM

Group Art Unit: 1652

Examiner: D. Ramirez

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SUBMISSION OF SUBSTITUTE DRAWINGS

Subject to the approval of the Examiner, please replace the originally filed drawings with the forty-four (44) sheets of substitute drawings filed herewith (Figures 1 through 44). If the corrected drawings for any reason are not in full compliance with the pertinent statutes and regulations, please so advise the undersigned.

If any fees are necessary for the submission of these corrected drawings, please charge our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,  
GARRETT & DUNNER, L.L.P.

Dated: March 10, 2003

By:

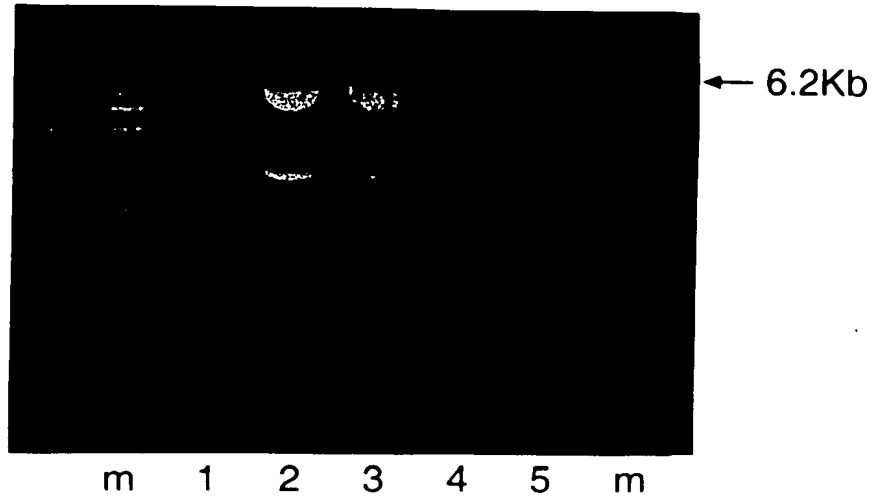
Robert W. Mann  
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FINNEGAN  
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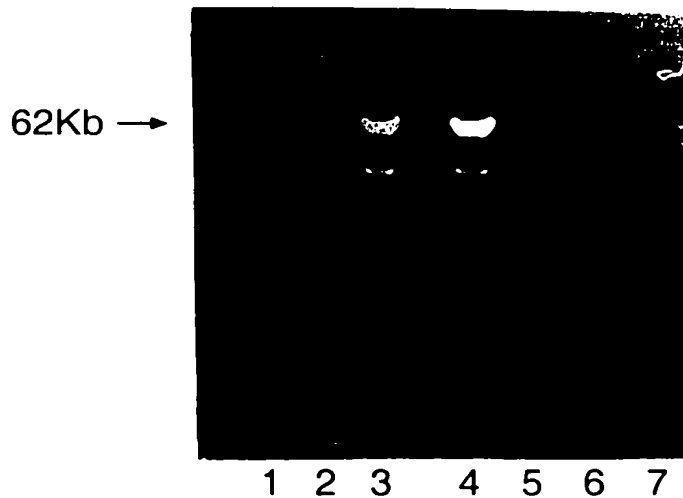
1300 I Street, NW  
Washington, DC 20005  
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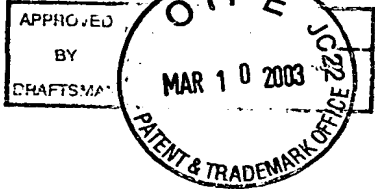
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 1 of 44



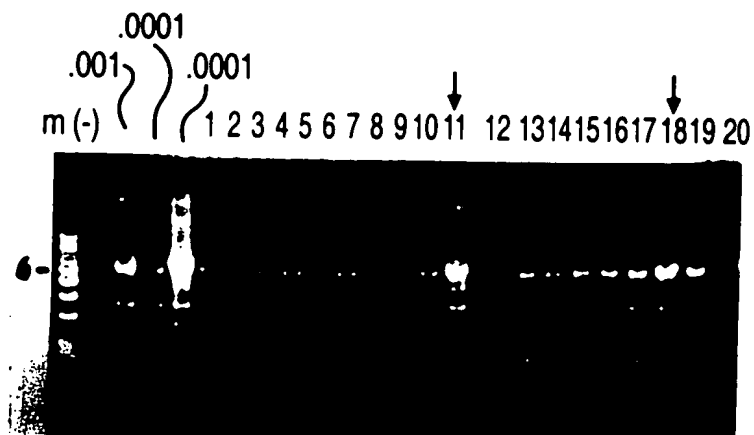
**FIG. 1**



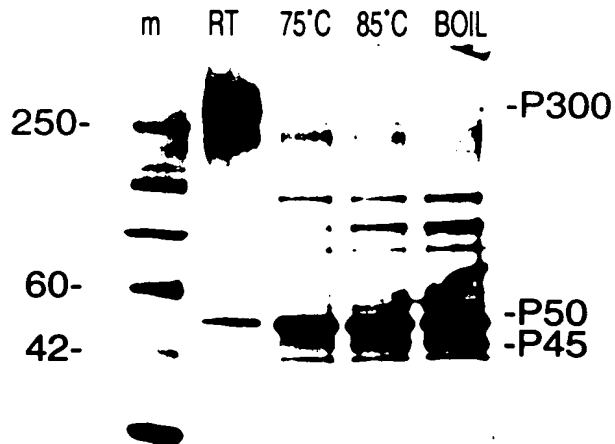
**FIG. 2**



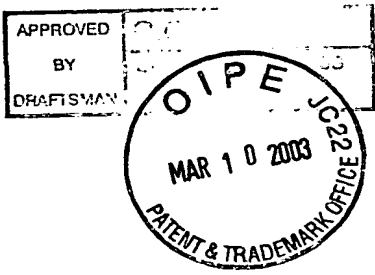
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS,  
PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND  
METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
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Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 2 of 44



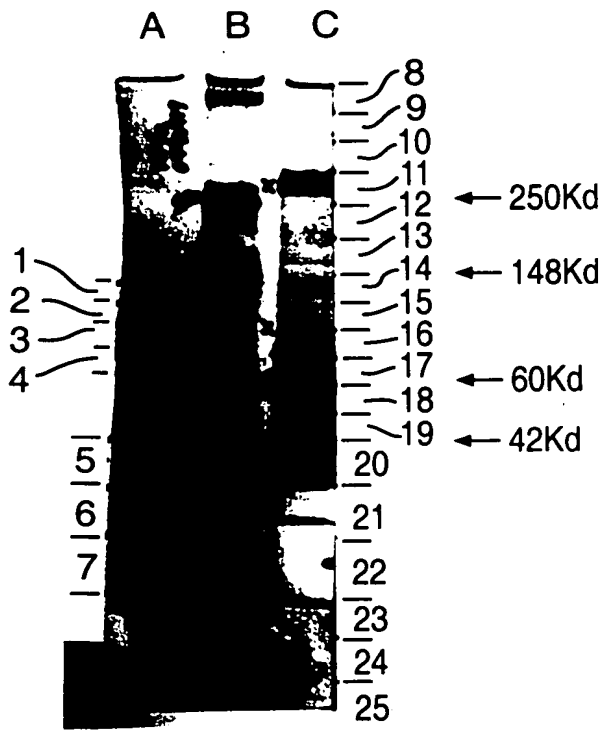
**FIG. 3**



**FIG. 7**

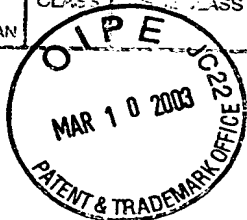


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Inventors: Holly H. HOGREFE et al.  
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Page 3 of 44

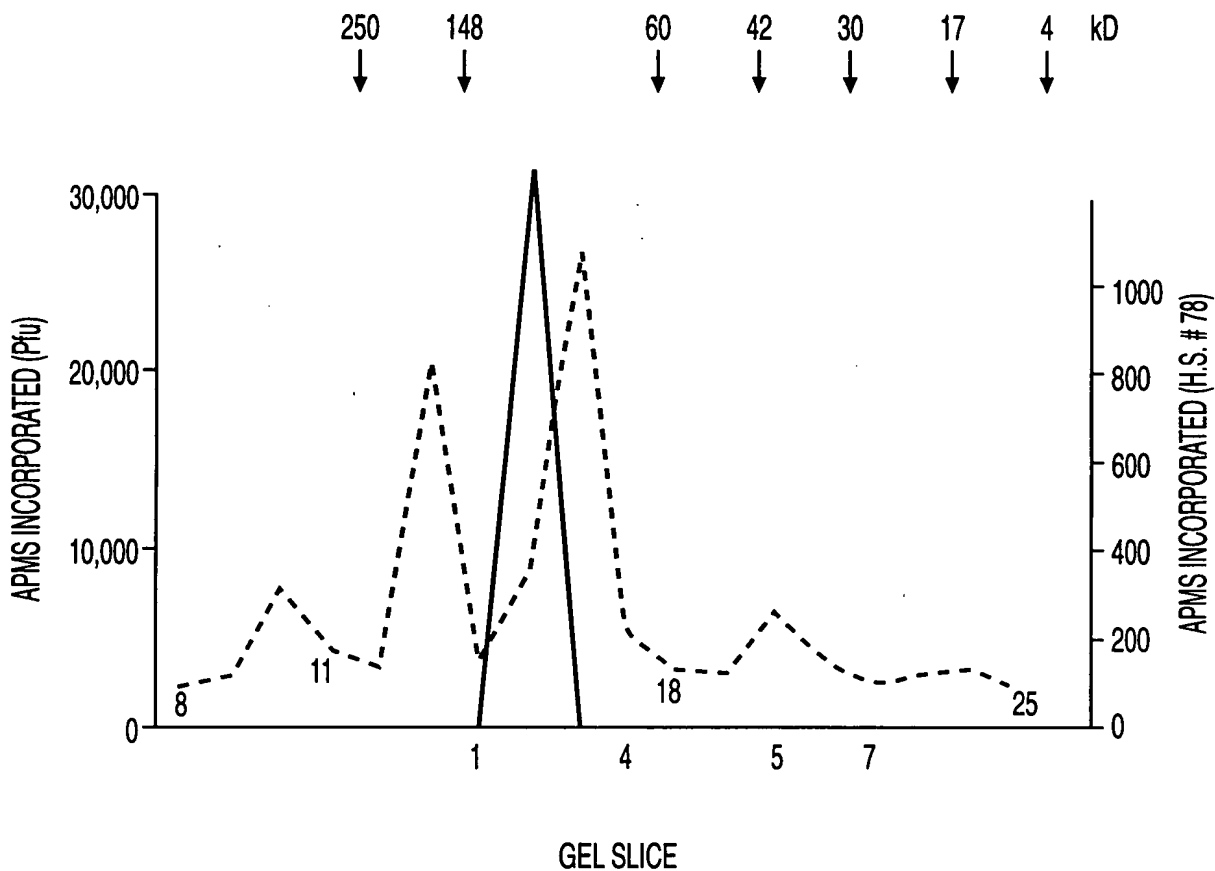


**FIG. 4**

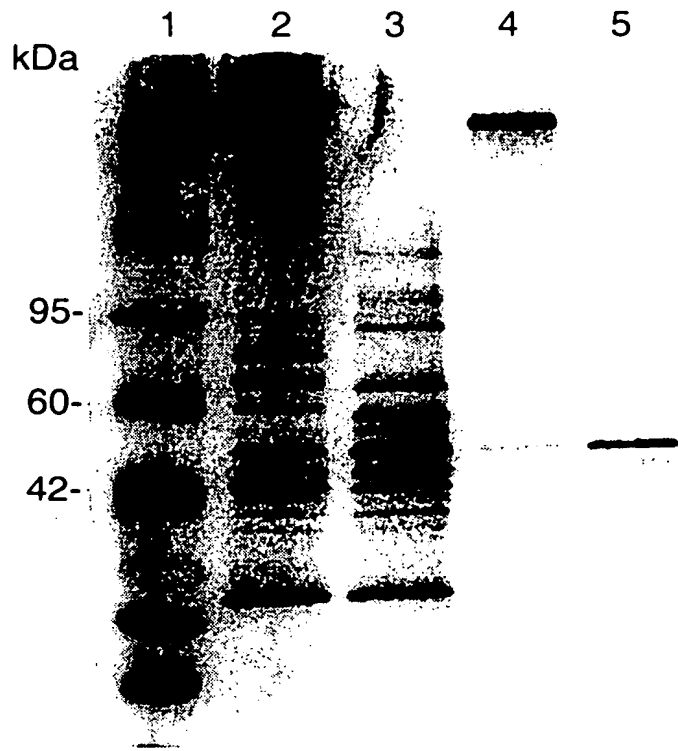
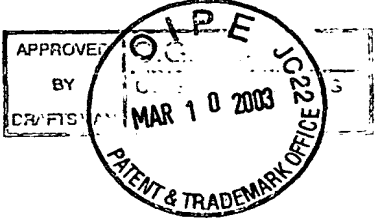
|           |                     |
|-----------|---------------------|
| APPROVED  | O.G. FIG.           |
| BY        | CLASS & CLASS CLASS |
| DRAFTSMAN |                     |



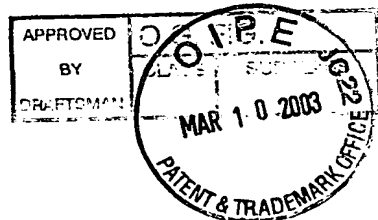
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
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 Page 4 of 44



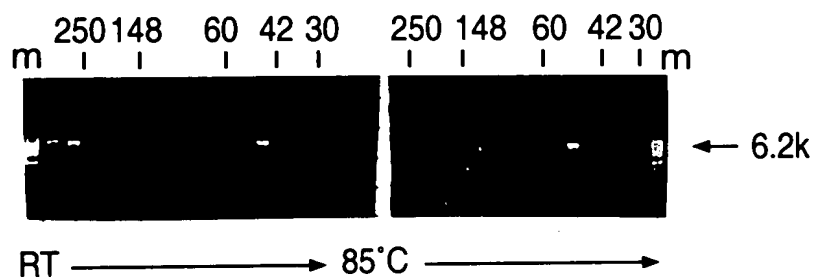
**FIG. 5**



**FIG. 6**



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
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Page 6 of 44



**FIG. 8**

APPROVED BY DRAFTSMAN  
O.G. F. CLASS. AGG.  
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Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 7 of 44

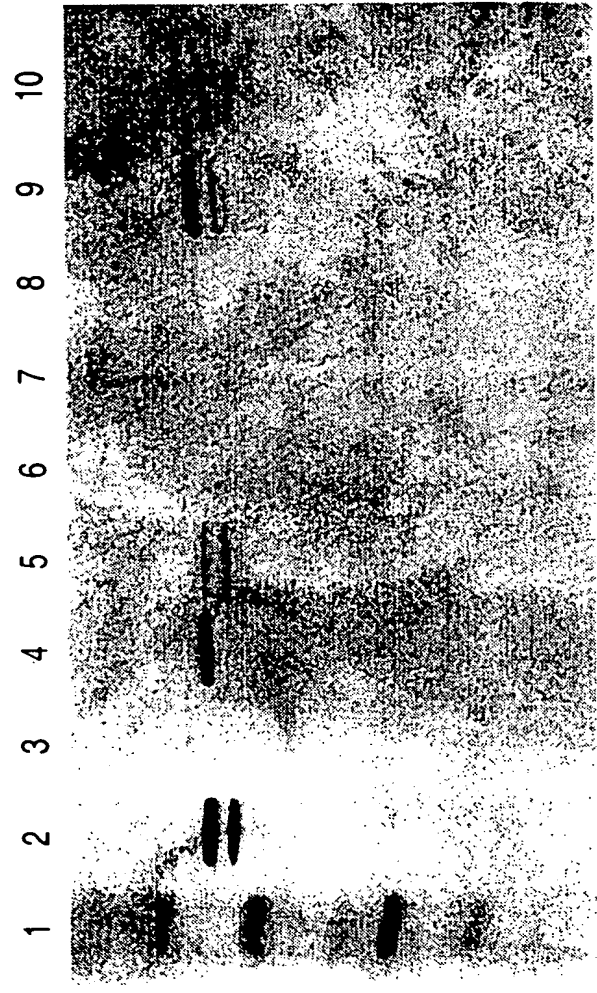


FIG. 9



|           |       |
|-----------|-------|
| APPROVED  | O.G.  |
| BY        | CLASS |
| DRAFTSMAN | ASS   |

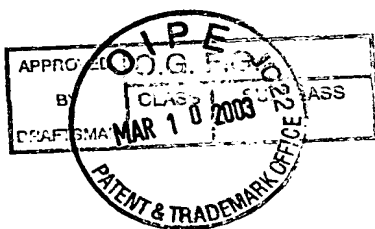
  

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Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
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 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 8 of 44



**FIG. 10**



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 9 of 44



**FIG. 11A**

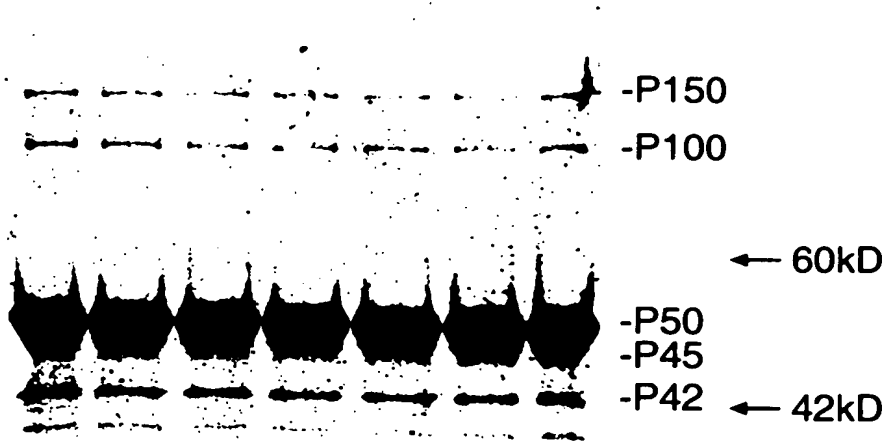


**FIG. 11B**

|           |           |          |
|-----------|-----------|----------|
| APPROVED  | J.G. FIG. |          |
| BY        | PLSSE     | SUBCLASS |
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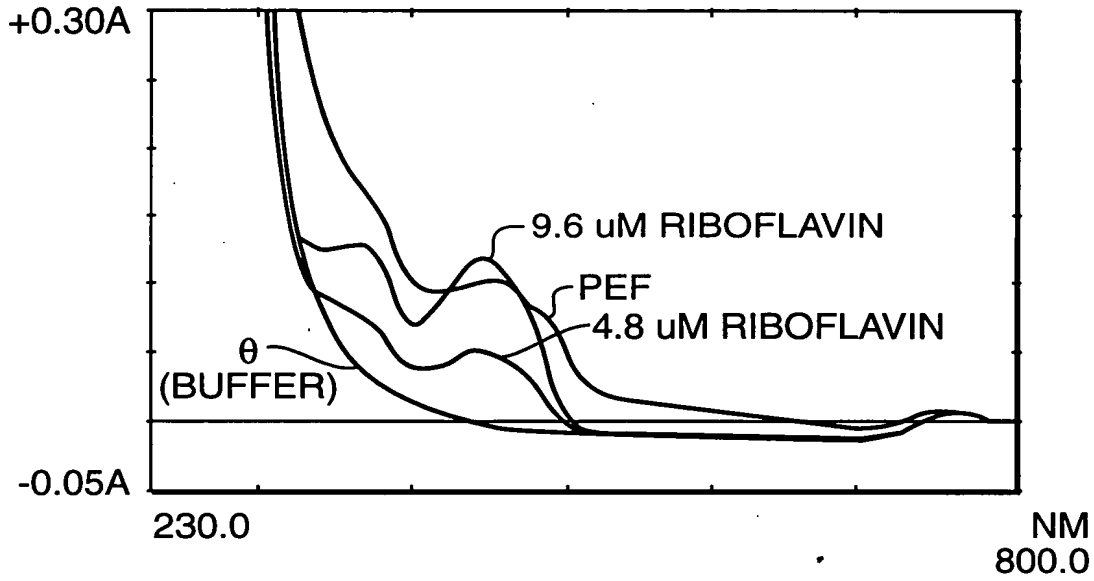
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
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Page 10 of 44



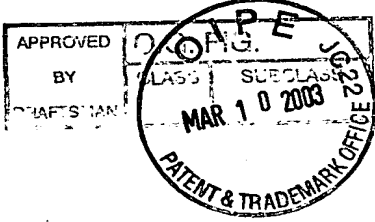
**FIG. 12**

APPROVED BY O.G.F. 301 P.E. SUBCLASS MAR 10 2003 PATENT & TRADEMARK OFFICE

Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING S  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 11 of 44



**FIG. 13**



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
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Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 12 of 44

|      |   |     | CLONE 1    |   |     | CLONE 23 |             |             |
|------|---|-----|------------|---|-----|----------|-------------|-------------|
|      |   |     | PEF LOT 49 |   |     |          |             |             |
| NONE | 7 | 0.7 | 0.2        | 7 | 0.7 | 0.2      | 1 ul (1/10) | 1 ul (1/10) |
|      |   |     |            |   |     |          | 5 ul        | 5 ul        |

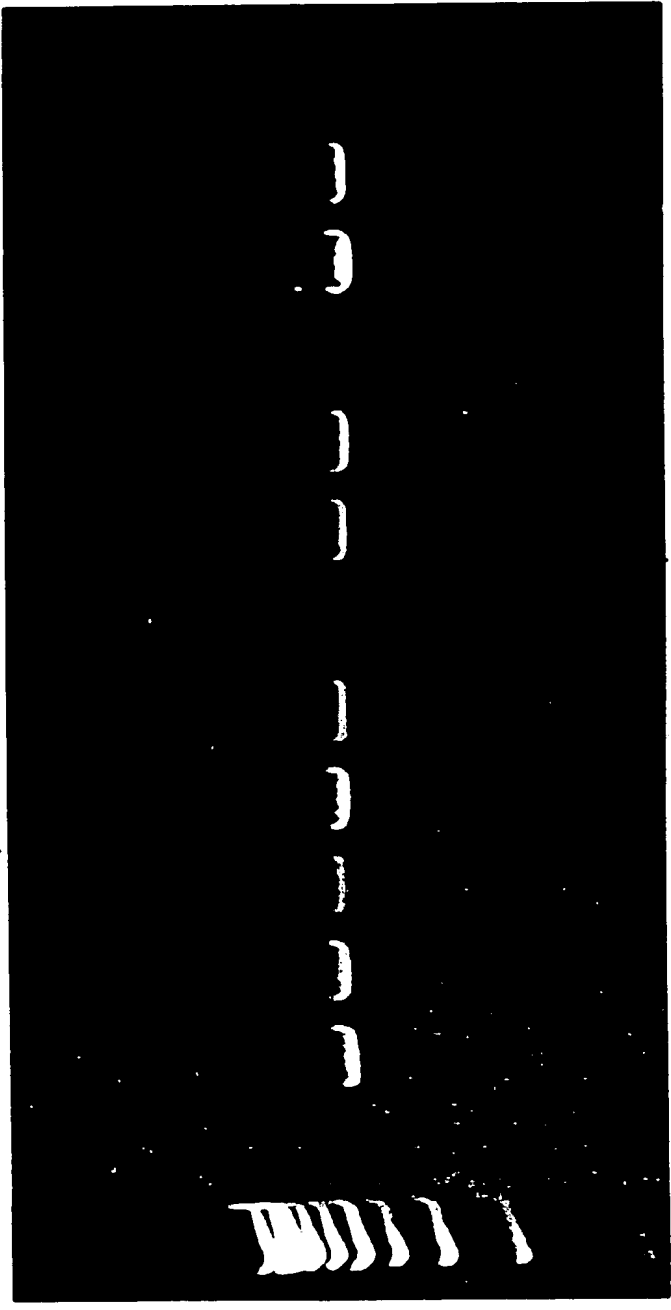
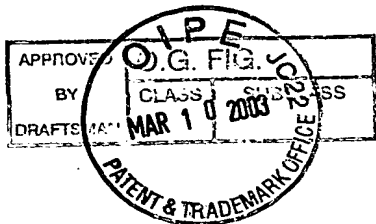
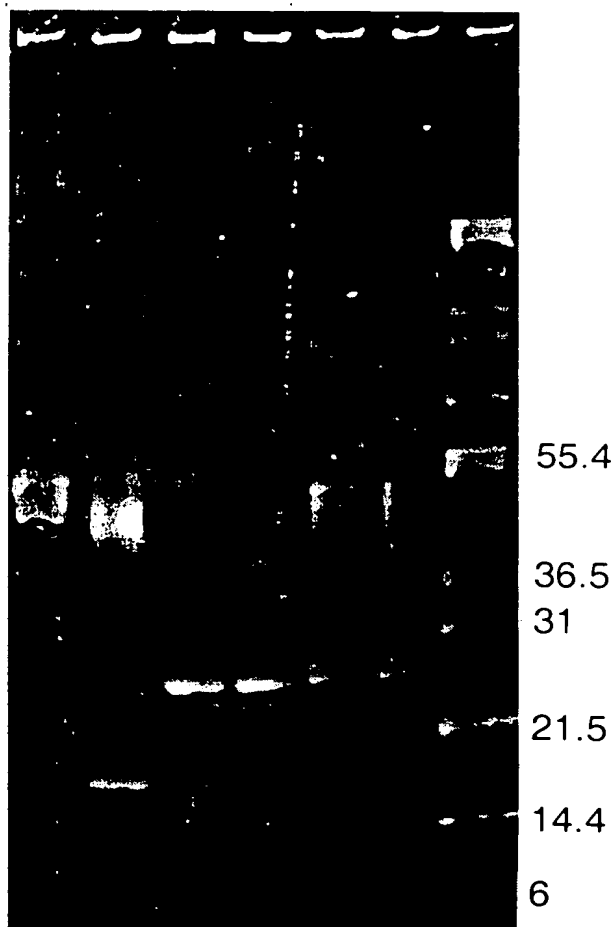


FIG. 14



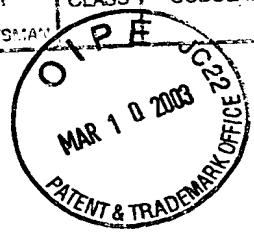
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING  
Inventors: Holly H. HOGREFE et al.  
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Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 13 of 44

|                 | <u>BOILED</u> | <u>UNBOILED</u> |
|-----------------|---------------|-----------------|
| 100 ng PEF      | rp45 CLONE 1  | rp45 CLONE 1    |
| BOILED W/ 1%TCA | {             | {               |
| 100 ng PEF      |               |                 |
|                 | rp45 CLONE 23 | rp45 CLONE 23   |

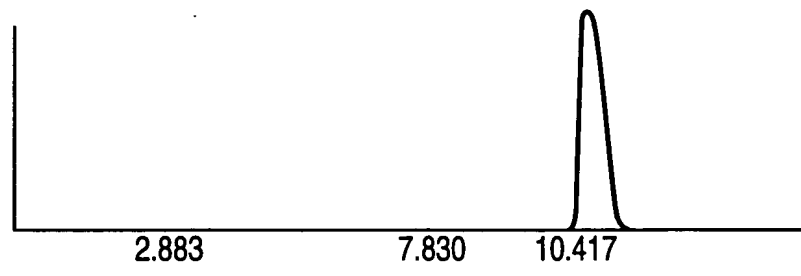


**FIG. 15**

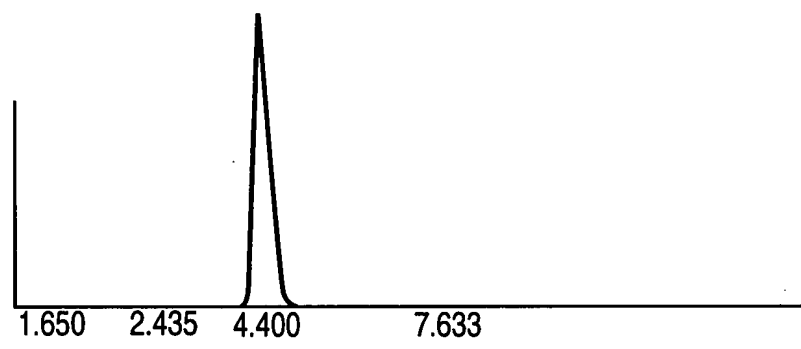
|           |           |          |
|-----------|-----------|----------|
| APPROVED  | O.G. FIG. |          |
| BY        | CLASS     | SUBCLASS |
| DRAFTSMAN |           |          |



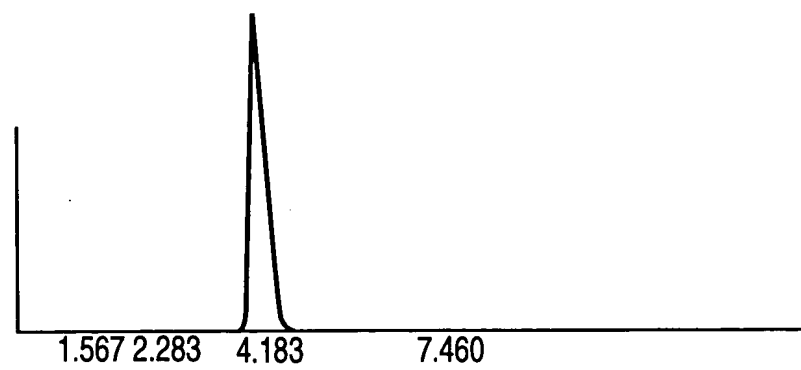
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 14 of 44



**FIG. 16A**



**FIG. 16B**

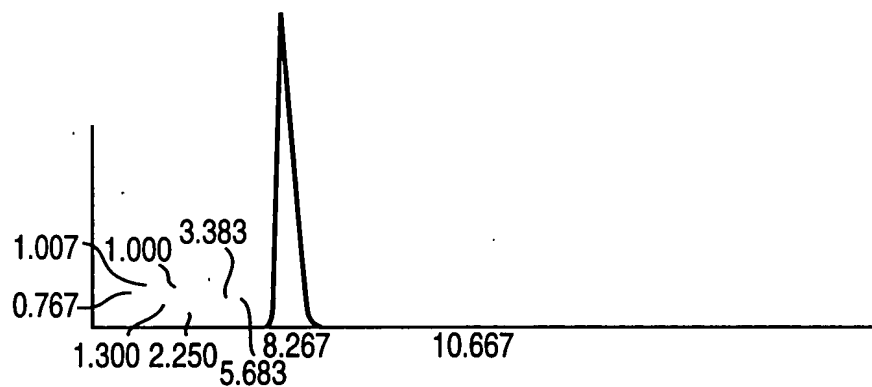


**FIG. 16C**

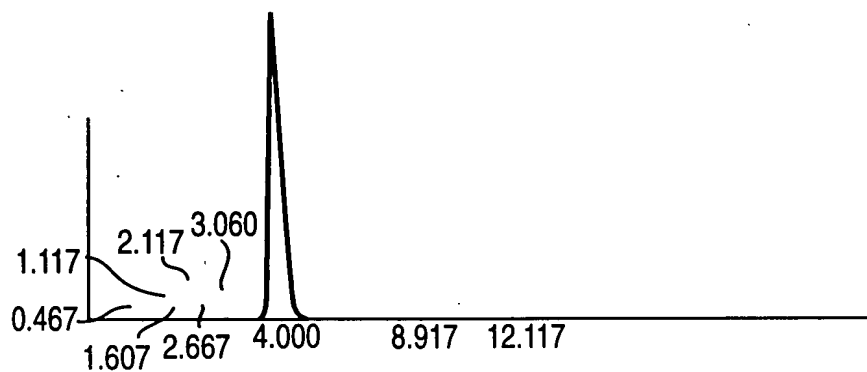
|           |           |          |
|-----------|-----------|----------|
| APPROVED  | D.G. FIG. |          |
| BY        | CLASS     | SUBCLASS |
| DRAFTSMAN |           |          |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
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 Application No.: 08/957,709  
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 Attorney Docket No.: 04121.0116-01000  
 Page 15 of 44

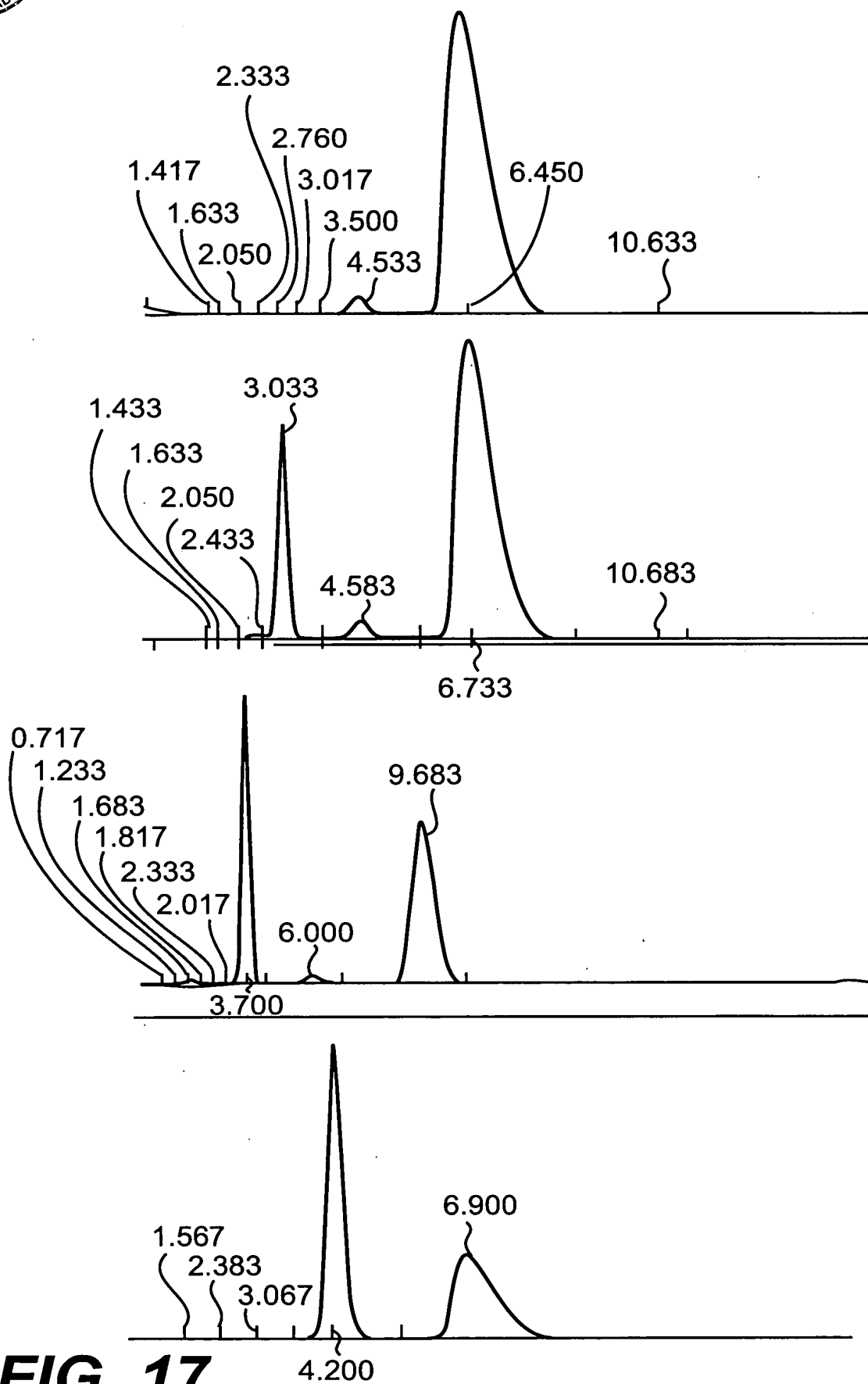
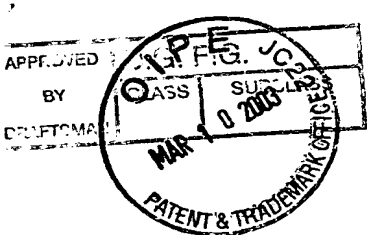


**FIG. 16D**

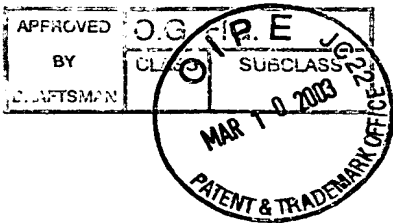


**FIG. 16E**

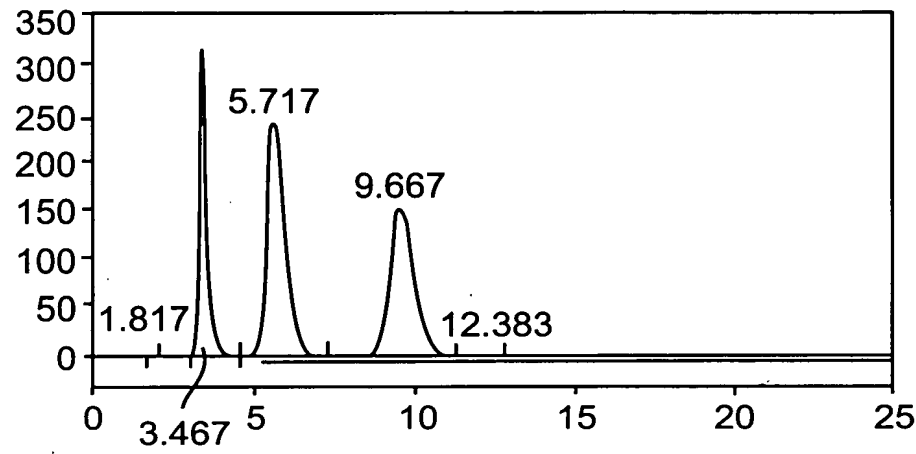




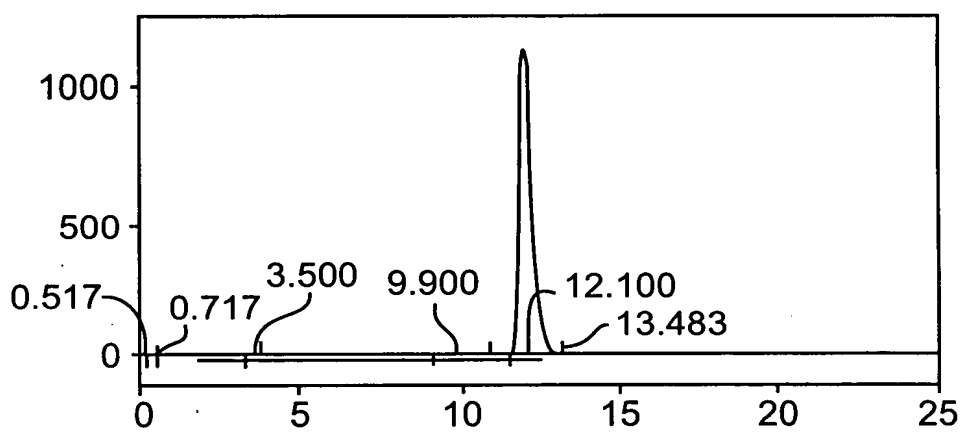
**FIG. 17**



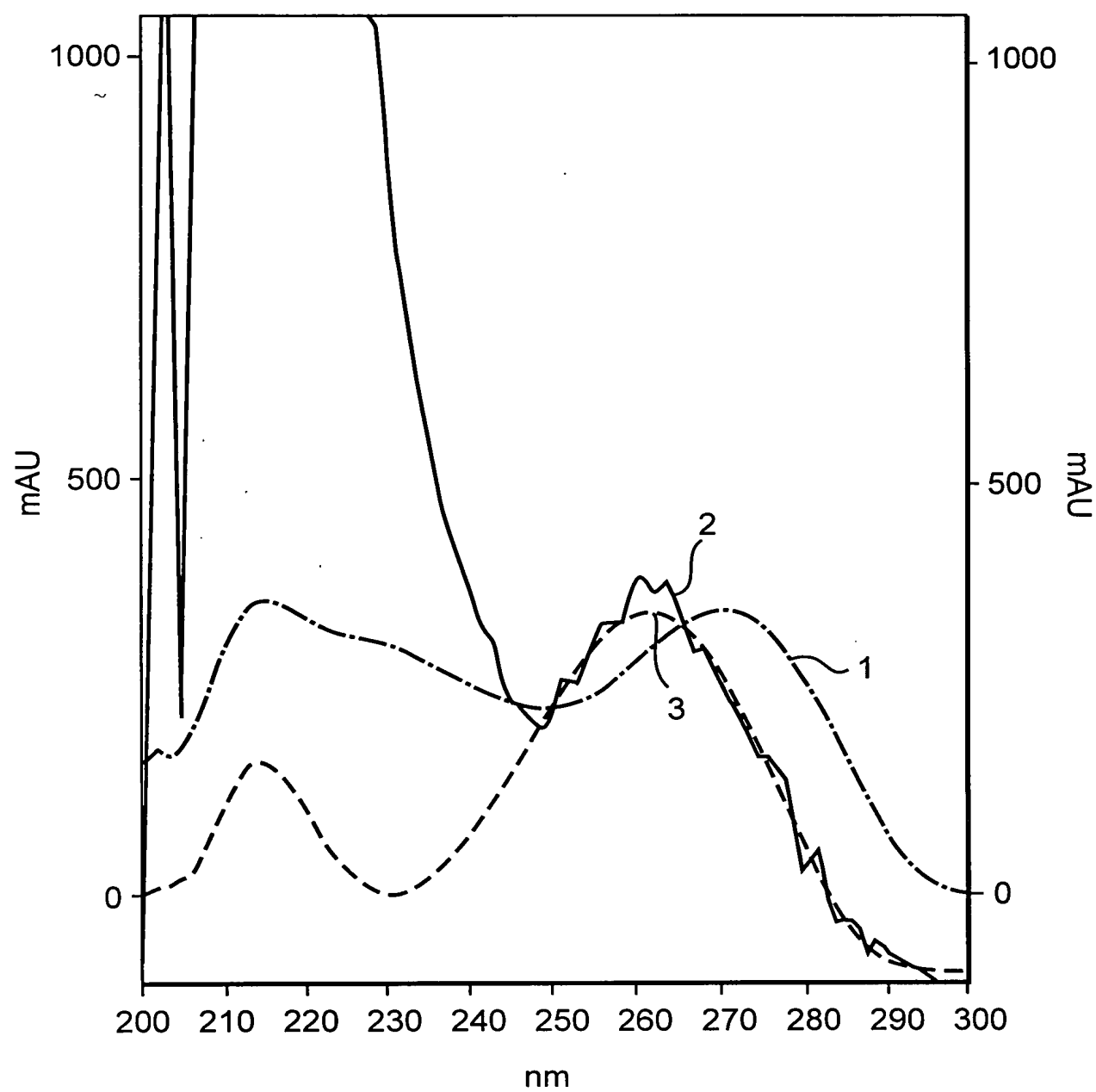
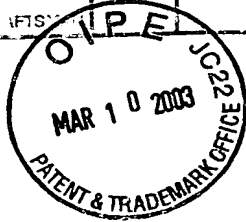
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 17 of 44



**FIG. 18C**

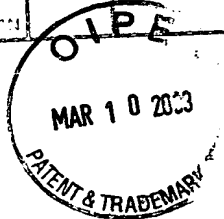


**FIG. 18D**

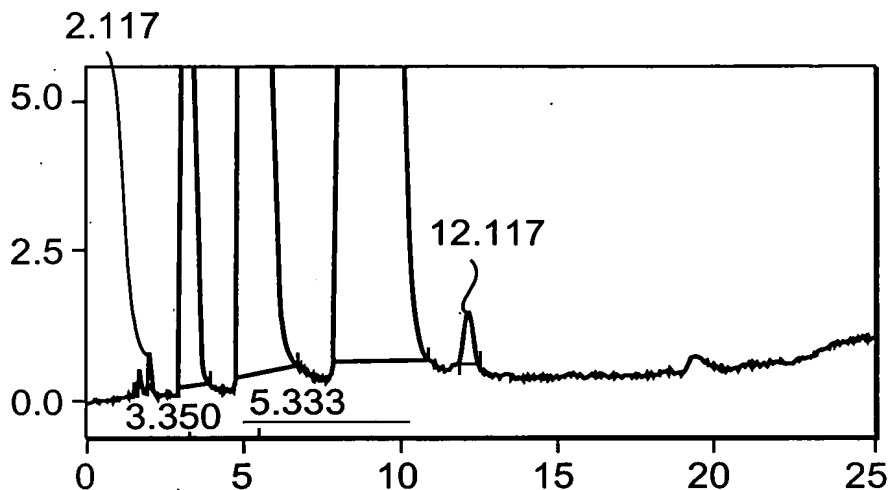


**FIG. 19**

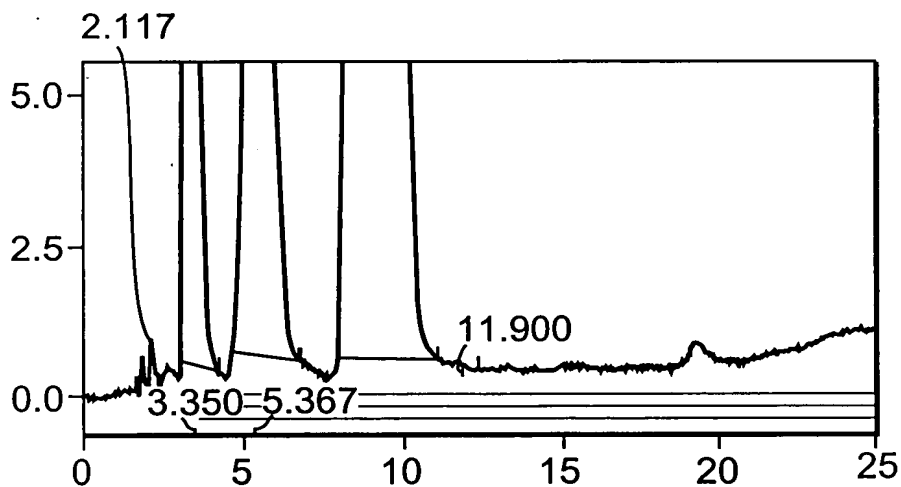
|           |           |          |
|-----------|-----------|----------|
| APPROVED  | J.C. FIG. |          |
| BY        | CLASS     | SUBCLASS |
| DRAFTSMAN |           |          |



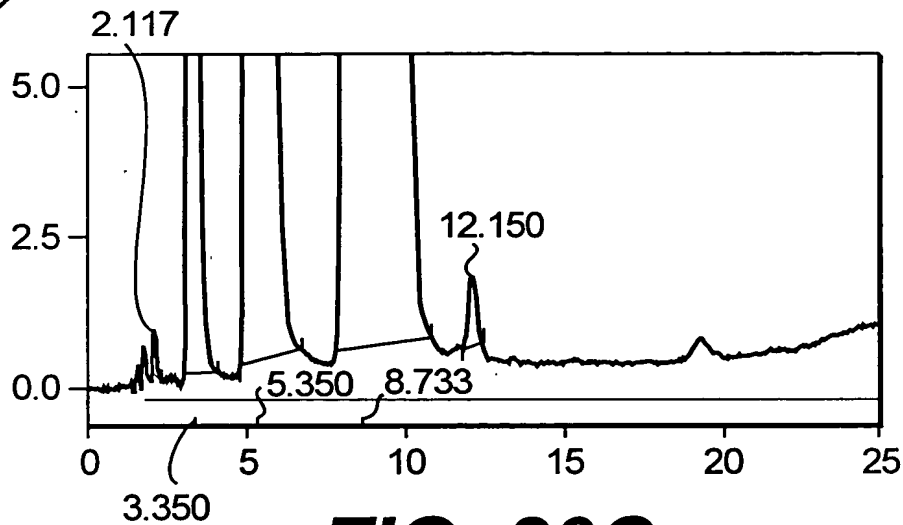
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 19 of 44



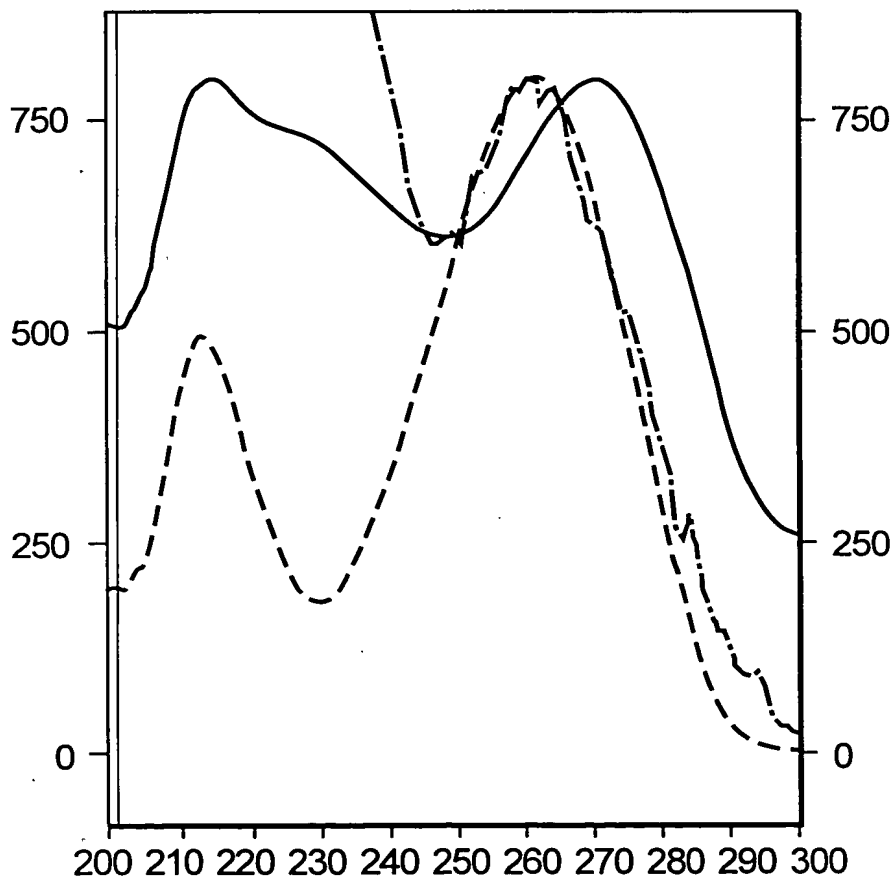
**FIG. 20A**



**FIG. 20B**



**FIG. 20C**



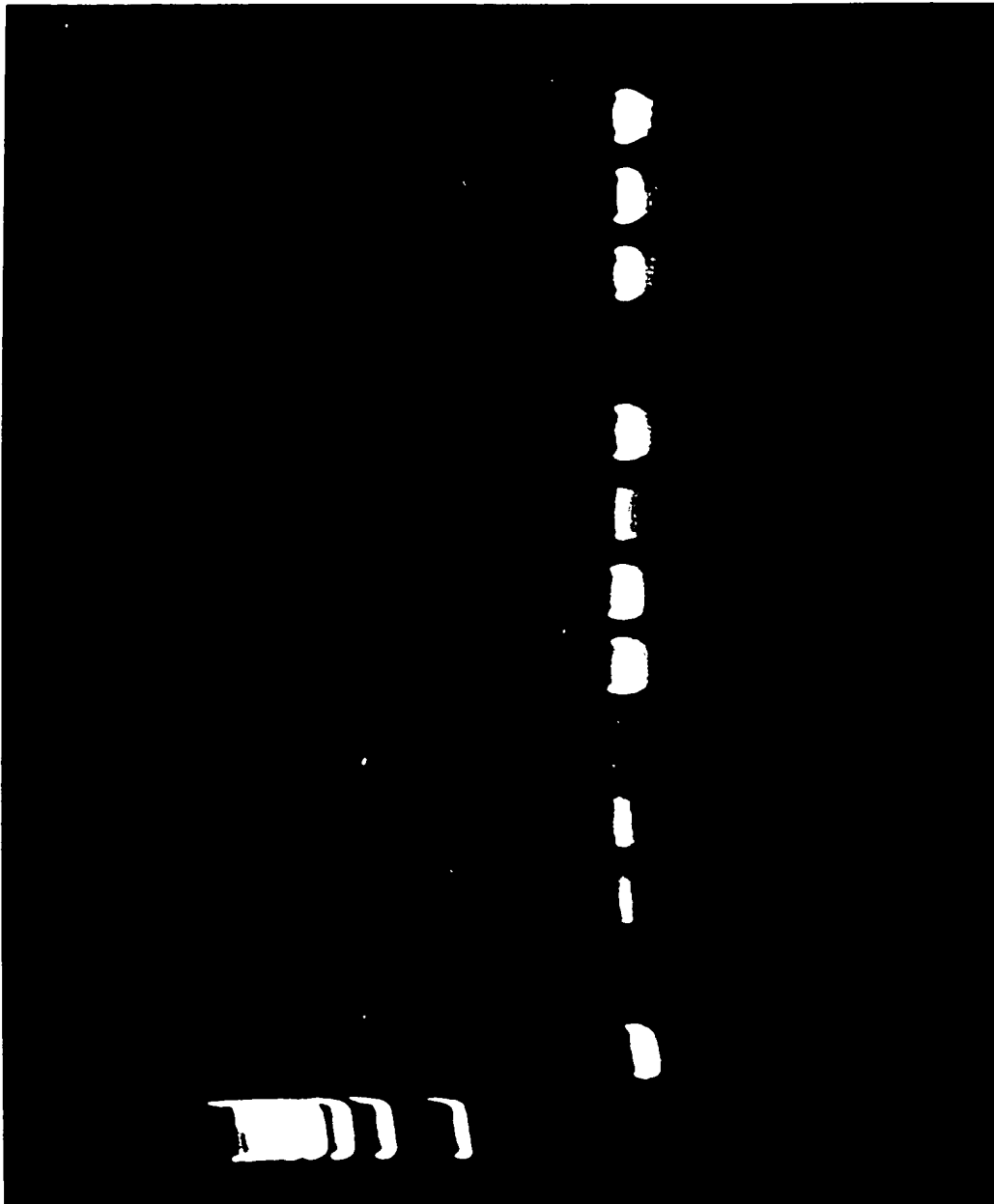
**FIG. 20D**

|           |          |
|-----------|----------|
| APPROVED  | FIG      |
| BY        | CLASS    |
| CHAFTSMAN | SUBCLASS |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
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 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 21 of 44

1 2 3 4 5 6 7 8 9 10 11 12 13



**FIG. 21**

|          |           |          |
|----------|-----------|----------|
| APPROVED | O.G. FIG. |          |
| BY       | CLASS     | SUBCLASS |
| DEFTSMAN |           |          |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
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 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 22 of 44

| Pfu Pol |                          |                                 | Taq Pol                   |
|---------|--------------------------|---------------------------------|---------------------------|
| NO      | Pmole CONTROL OLIGO      | Pmole dU OLIGO                  | Pmole dU OLIGO            |
| PEF     | 16 8 1.6 0.8 0.16 M 0.08 | 0.016<br>16 8 1.6 0.8 0.16 0.08 | 0.16<br>16 8 1.6 0.8 0.08 |
| -       |                          |                                 |                           |
| +       |                          |                                 |                           |

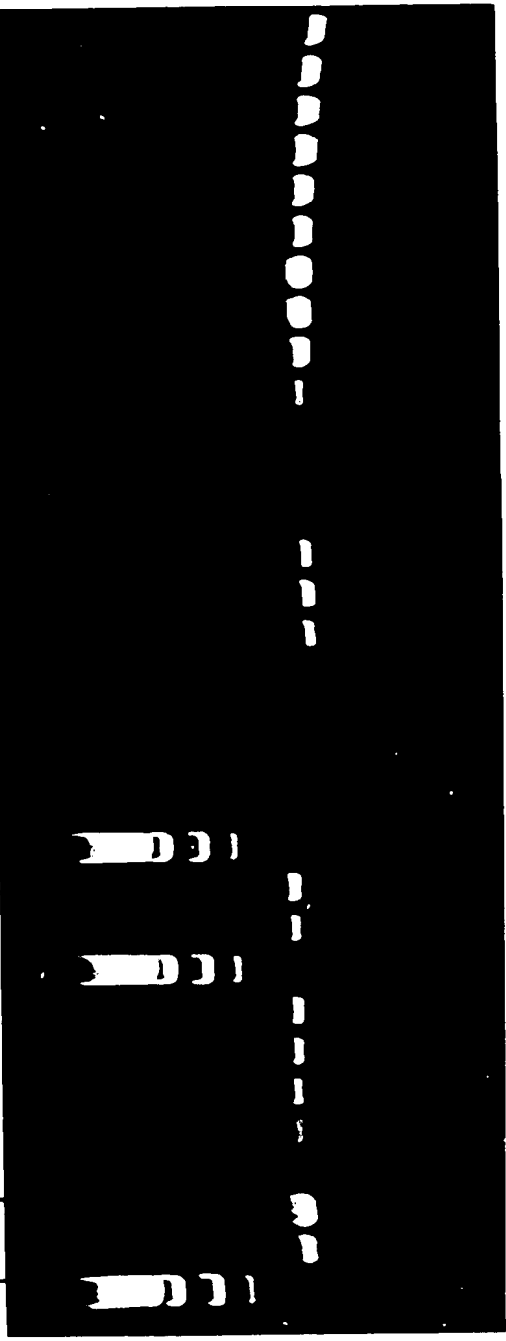


FIG. 22

|          |           |          |
|----------|-----------|----------|
| APPROVED | O.G. FIG. |          |
| BY       | CLASS     | SUBCLASS |
| DEPTSMAN |           |          |



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Attorney Docket No.: 04121.0116-01000  
Page 23 of 44

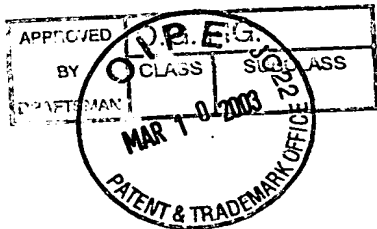
|        |              |       |           |          |
|--------|--------------|-------|-----------|----------|
| 1 Kb   | NEGATIVE     | BLANK | HdUTPase  | HdUTPase |
| LADDER | } POSITIVE } |       | UNDILUTED | 1/10     |

5kb →



**FIG. 23**





Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
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Application No.: 08/957,709  
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Page 24 of 44

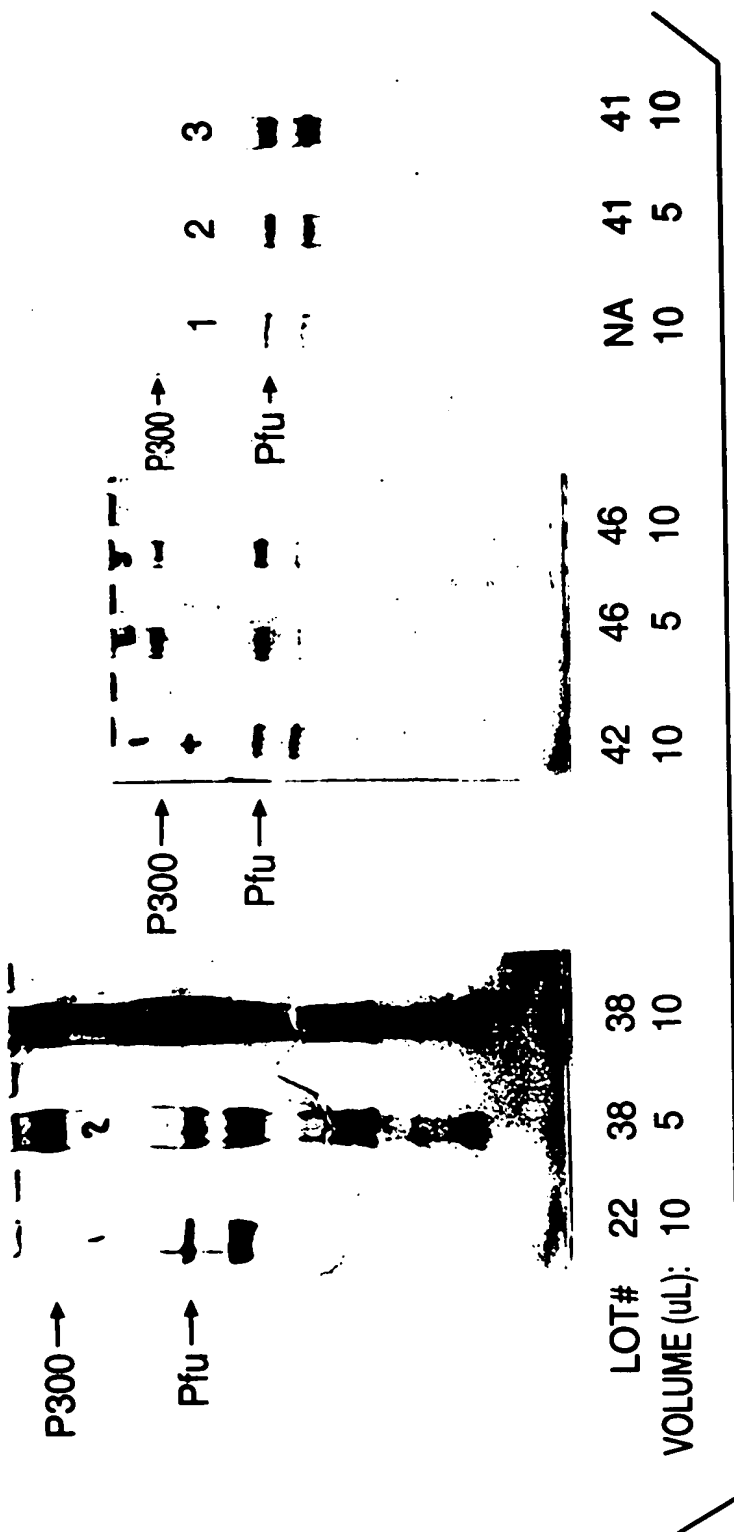
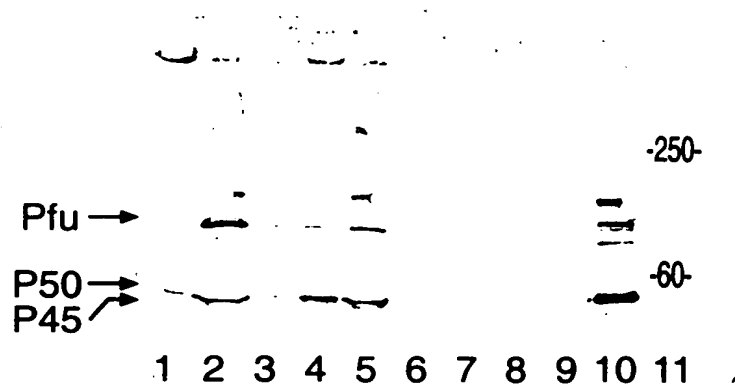


FIG. 24

|          |           |          |
|----------|-----------|----------|
| APPROVED | O.G. FIG. |          |
| BY       | CLASS     | SUBCLASS |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING S  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
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 Page 25 of 44



**FIG. 25**

|           |           |          |
|-----------|-----------|----------|
| APPROVED  | O.G. FIG. |          |
| BY        | CLASS     | SUBCLASS |
| DRAFTSMAN |           |          |

**PIPE**

**MAR 10 2003**

**PATENT & TRADEMARK OFFICE 232**

Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING

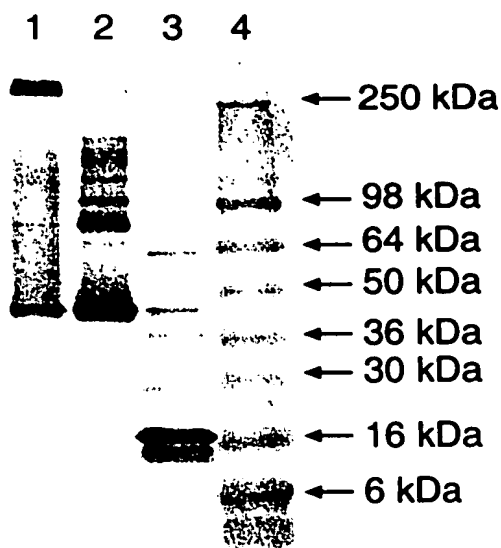
Inventors: Holly H. HOGREFE et al.

Application No.: 08/957,709

Filed: October 24, 1997

Attorney Docket No.: 04121.0116-01000

Page 26 of 44



**FIG. 26**

|           |           |          |
|-----------|-----------|----------|
| APPROVED  | O.O. FIG. |          |
| BY        | CLASS     | SUBCLASS |
| DRAFTSMAN |           |          |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 27 of 44

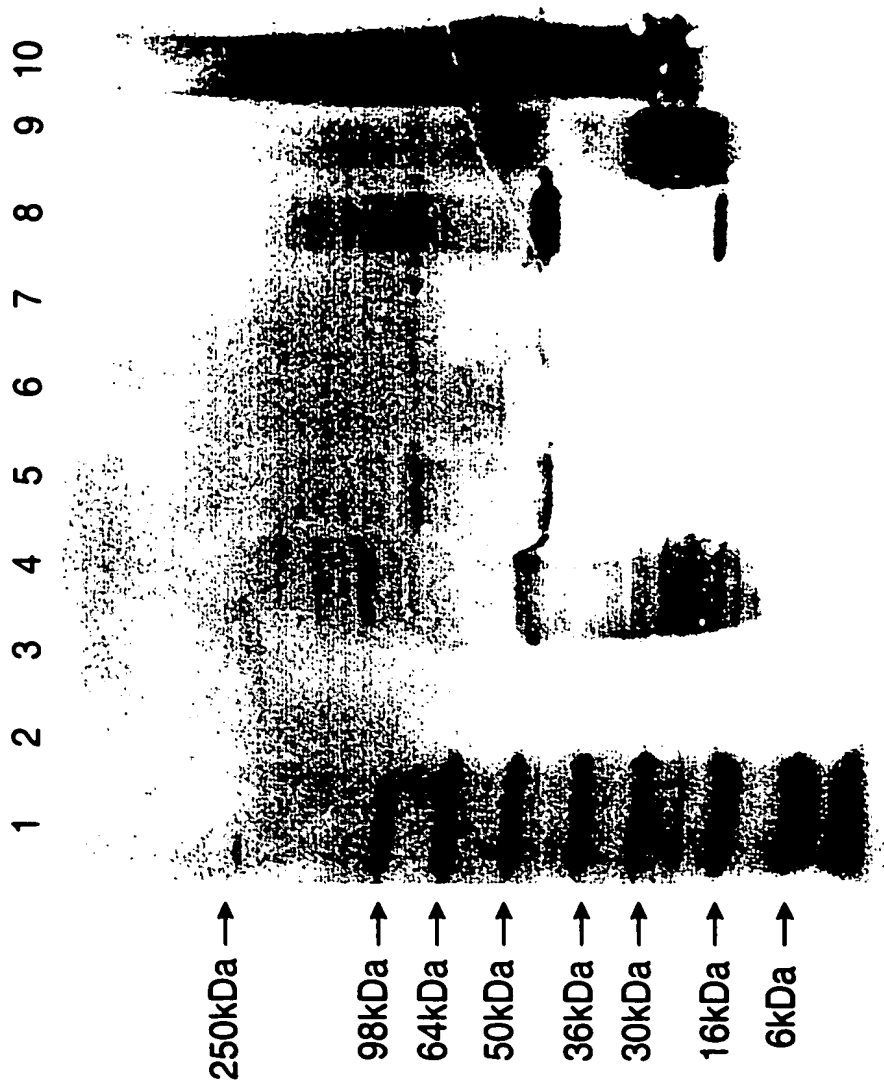
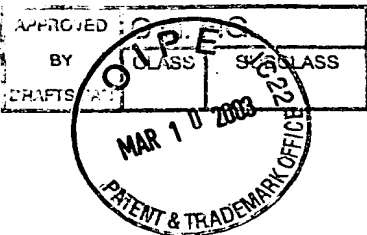


FIG. 27



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SA  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 28 of 44

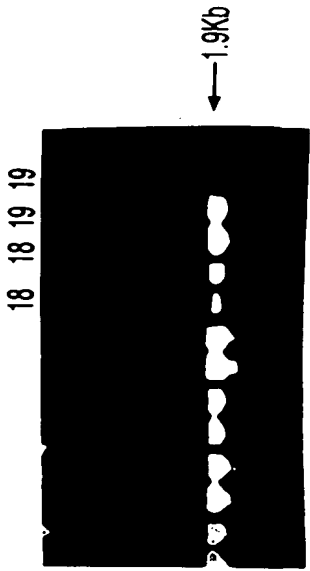
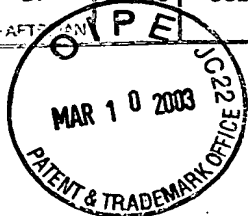
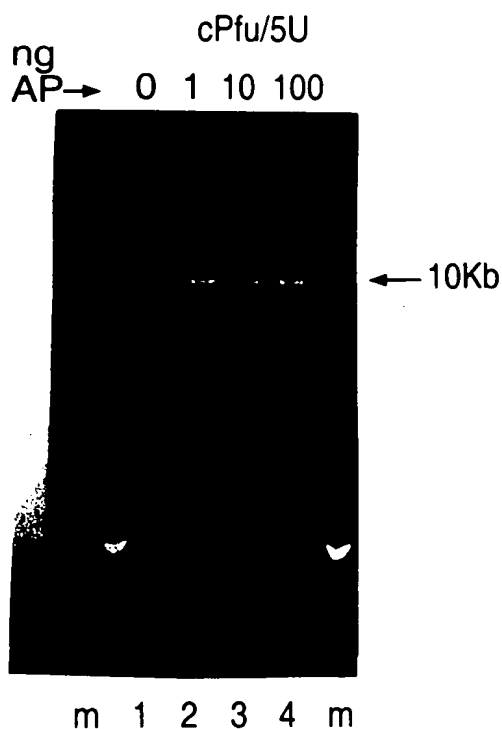


FIG. 28

|          |           |          |
|----------|-----------|----------|
| APPROVED | O.G. FIG. |          |
| BY       | CLASS     | SUBCLASS |
| DATE     |           |          |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 29 of 44

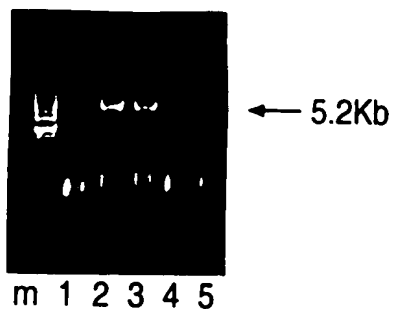


**FIG. 29**

|           |       |          |
|-----------|-------|----------|
| INVENTOR  | CLASS | SUBCLASS |
| BY        | CLASS | SUBCLASS |
| CRAFTSMAN |       |          |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS,  
PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND  
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Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 30 of 44

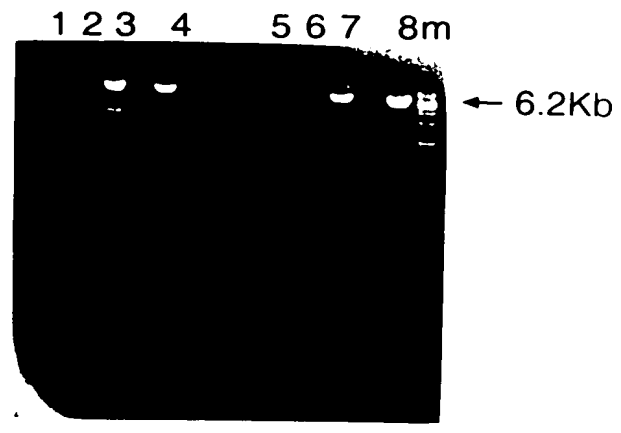


**FIG. 30**

BY CLASS SUBCLASS



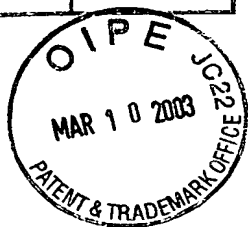
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 31 of 44



**FIG. 31**



|           |           |          |
|-----------|-----------|----------|
| APPROVED  | D.G. H.G. |          |
| BY        | CLASS     | SUBCLASS |
| DRAFTSMAN |           |          |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 32 of 44

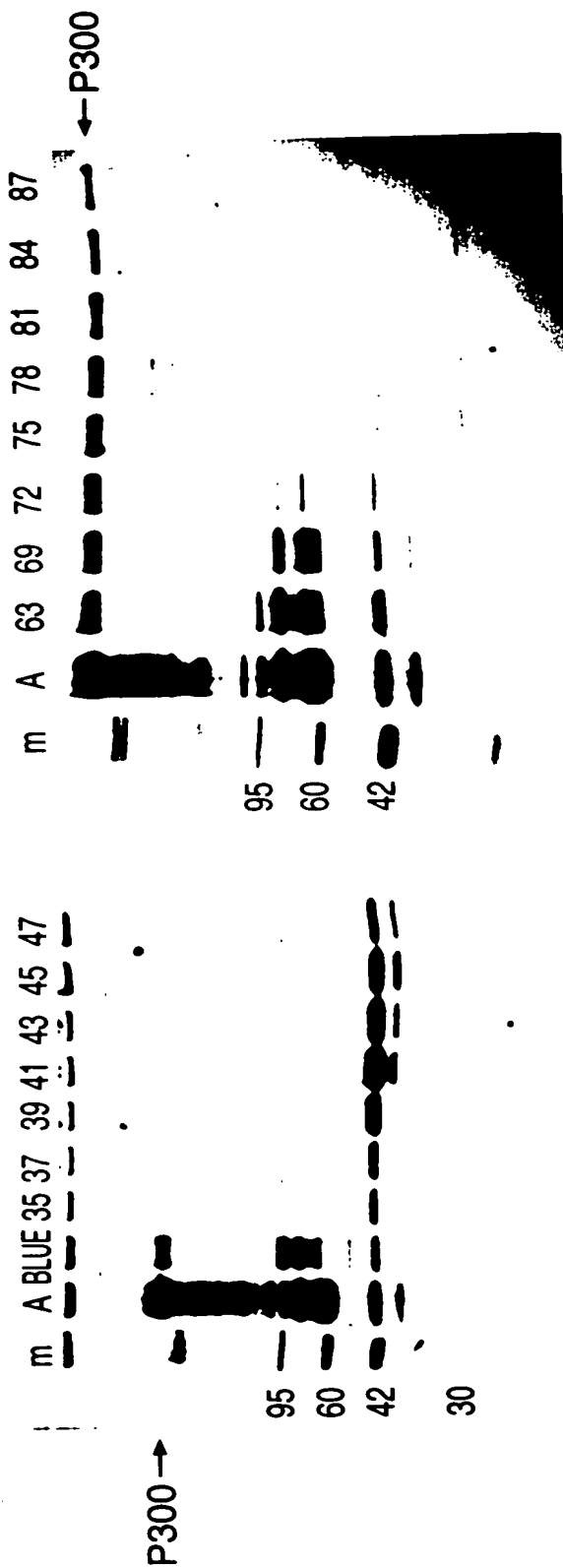


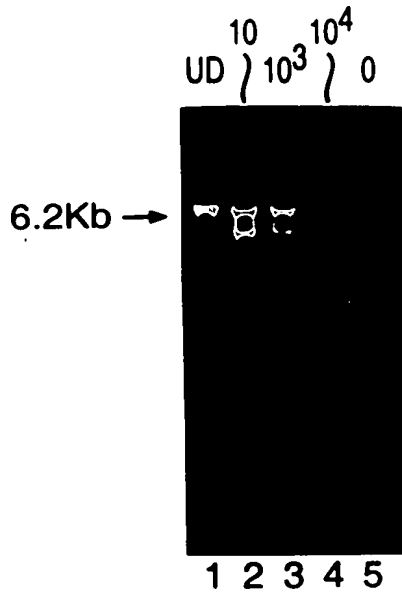
FIG. 32A

FIG. 32B

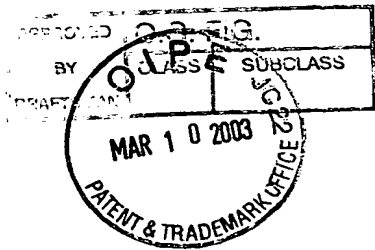
|           |           |          |
|-----------|-----------|----------|
| APPROVED  | O.G. FIG. |          |
| BY        | CLASS     | SUBCLASS |
| DRAFTSMAN |           |          |



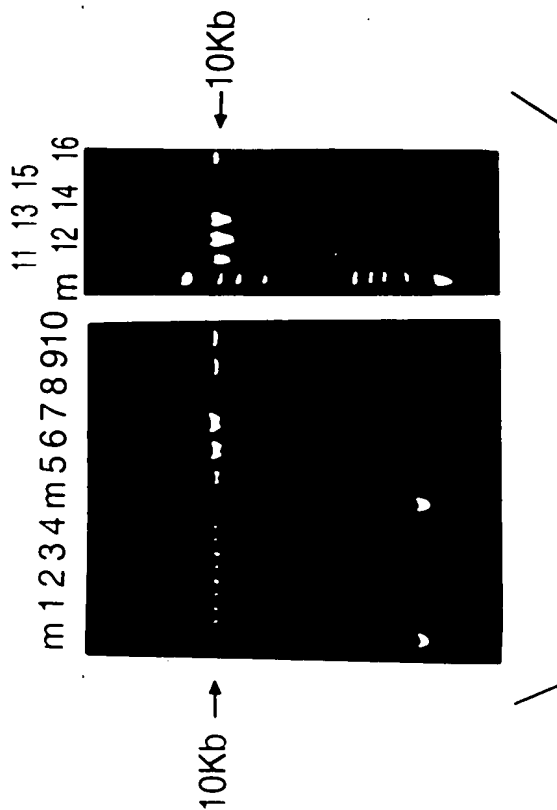
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 33 of 44



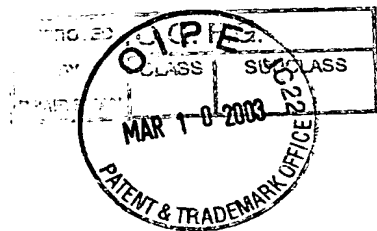
**FIG. 33**



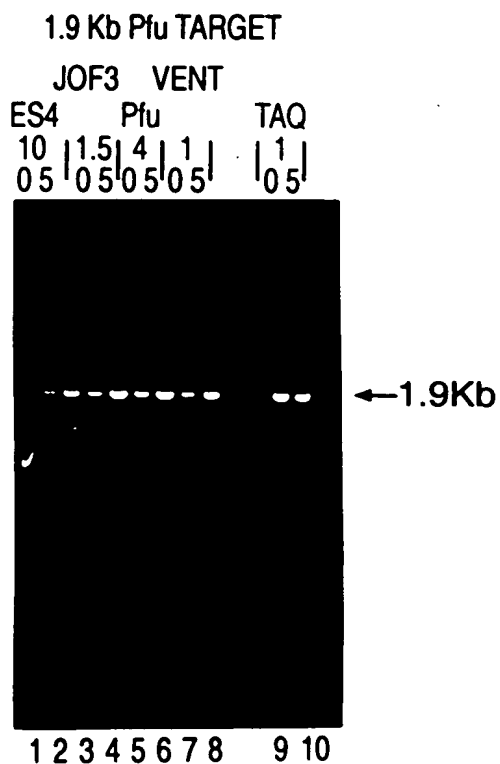
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 34 of 44



**FIG. 34**

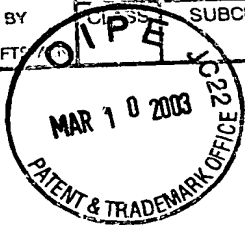


Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS,  
PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND  
METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 35 of 44



**FIG. 35**

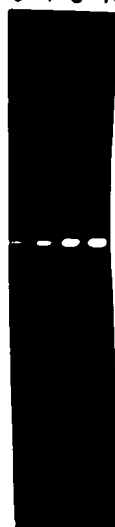
|          |                |
|----------|----------------|
| APPROVED | O.G. FIG.      |
| BY       | CLASS SUBCLASS |
| DRAFT    |                |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS,  
PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND  
METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 36 of 44

DEEP VENT USING 0.75u

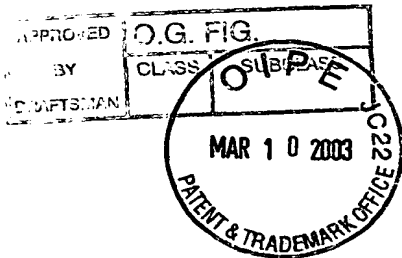
0 1 5 10



← 2Kb

1 2 3 4

**FIG. 36**



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SA  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 37 of 44

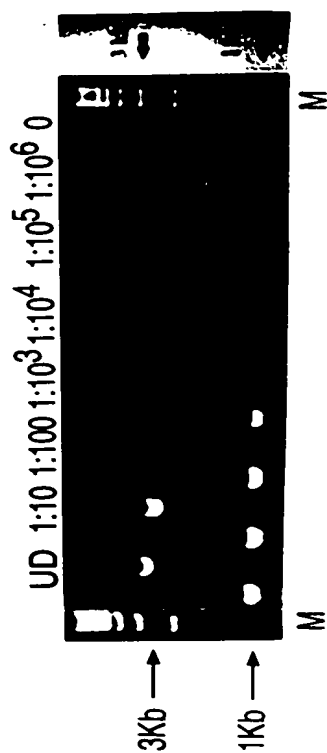
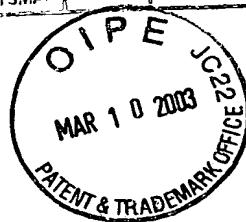
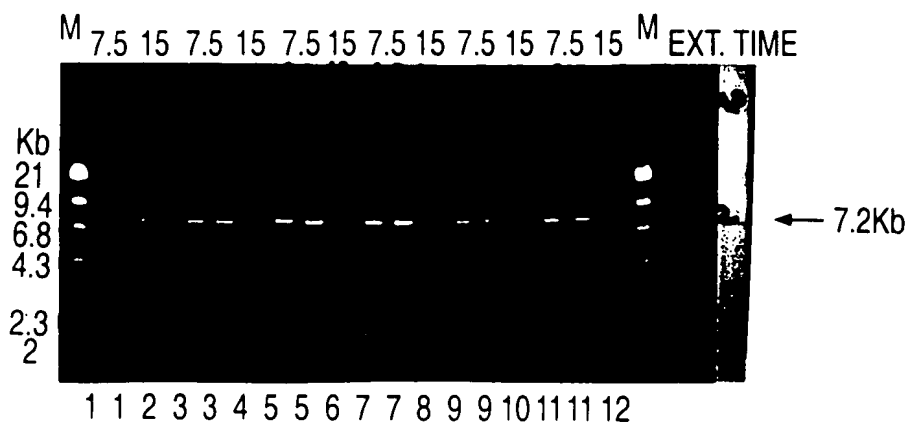


FIG. 37

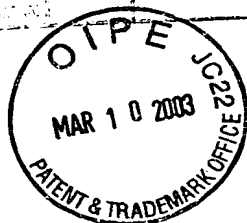
|                           |              |          |
|---------------------------|--------------|----------|
| APPROVED                  | U.S. G. FIG. |          |
| BY                        | CLASS        | SUBCLASS |
| PATENT & TRADEMARK OFFICE |              |          |



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 38 of 44



**FIG. 38**



## Pfu "PCR ENHANCER" IN QUICKCHANGE MUTAGENESIS

| LINEAR AMPLIFICATION<br>CONDITIONS |        |              |                | TRANSFORMATION<br>RESULTS |          |
|------------------------------------|--------|--------------|----------------|---------------------------|----------|
| POL.                               | BUFFER | H.S.<br>#75  | AMPL.<br>PROD. | #cfu                      | %MUTANTS |
| nPfu<br>(#38)                      | nPfu   | 0            | +              | 84                        | 95       |
|                                    |        | 1 $\mu$ 1    | $\pm$          | 47                        | 87       |
|                                    |        | .1 $\mu$ 1   | +              | 154                       | 97       |
|                                    |        | .01 $\mu$ 1  | ++             | 632                       | 95       |
|                                    |        | .001 $\mu$ 1 | +              | 484                       | 94       |
| nPfu<br>(#24A)                     | nPfu   | 0            | +              | 94                        | 89       |
|                                    |        | 1 $\mu$ 1    | +              | 34                        | 85       |
|                                    |        | .1 $\mu$ 1   | +              | 173                       | 91       |
|                                    |        | .01 $\mu$ 1  | ++             | 468                       | 96       |
|                                    |        | .001 $\mu$ 1 | +              | 230                       | 90       |

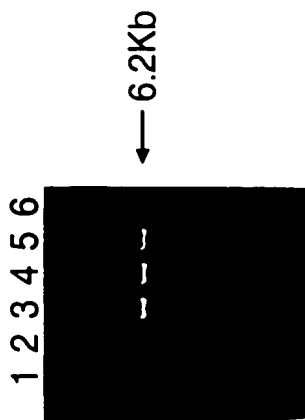
**FIG. 39**



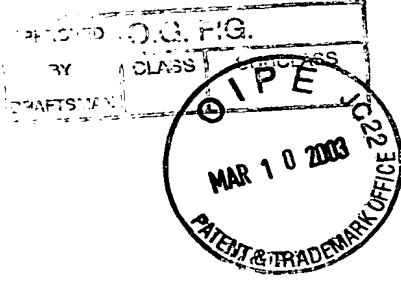
|               |           |          |
|---------------|-----------|----------|
| APPROVED      | O.C. FIG. |          |
| BY            | CLASS     | SUBCLASS |
| CONTACTS: JAM |           |          |



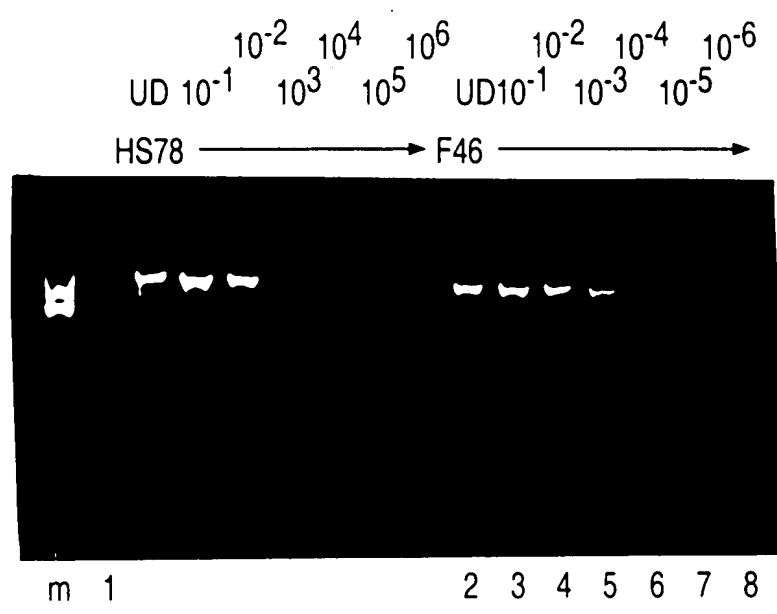
Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 40 of 44



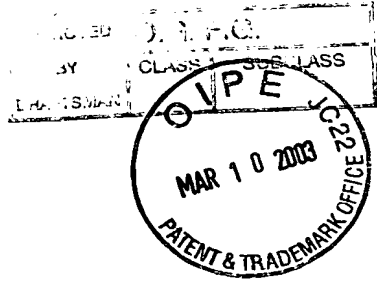
**FIG. 40**



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 41 of 44



**FIG. 41**



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SA  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 42 of 44

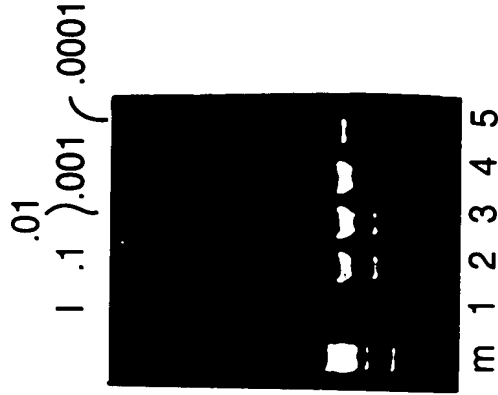
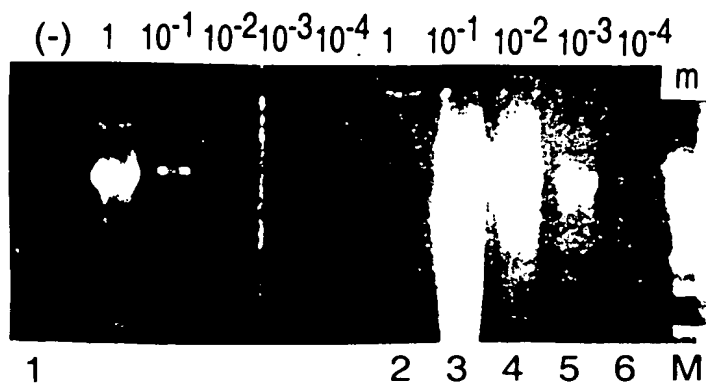


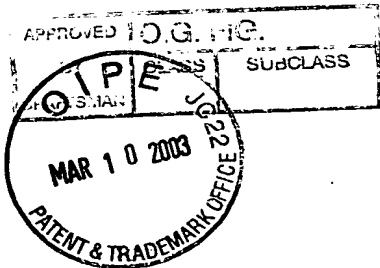
FIG. 42

|          |            |          |
|----------|------------|----------|
| APPROVED | O. B. FIG. |          |
| BY       | CLASS      | SUBCLASS |
| DATE     |            |          |

Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS, PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND METHODS FOR PURIFYING AND IDENTIFYING SAME  
 Inventors: Holly H. HOGREFE et al.  
 Application No.: 08/957,709  
 Filed: October 24, 1997  
 Attorney Docket No.: 04121.0116-01000  
 Page 43 of 44



**FIG. 43**



Title: POLYMERASE ENHANCING FACTOR (PEF) EXTRACTS,  
PEF PROTEIN COMPLEXES, ISOLATED PEF PROTEIN, AND  
METHODS FOR PURIFYING AND IDENTIFYING SAME  
Inventors: Holly H. HOGREFE et al.  
Application No.: 08/957,709  
Filed: October 24, 1997  
Attorney Docket No.: 04121.0116-01000  
Page 44 of 44



**FIG. 44B**



**FIG. 44A**